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Associations between Prenatal Maternal Stress and Birth Outcomes
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Associations between Prenatal Maternal Stress and Birth Outcomes

By

Huaiyu Zhang
M.S., University of Southern California, 2004

Advisor: Eugene Emory, Ph.D.

An Abstract of
A thesis submitted to the Faculty of the Graduate School of Emory University
in partial fulfillment of the requirements for the degree of
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Abstract

Associations between Prenatal Maternal Stress and Birth Outcomes
By Huaiyu Zhang

The literature on relationships between maternal prenatal stress and birth outcomes is mixed, partly attributable to the difference in operationalization of stress and birth outcome measures. This study aimed at applying a multivariate approach to assess these associations. Life event stress, perceived stress, and cortisol were included as stress indicators. Birth weight, gestational age, and Obstetric Complications Scale (OCS) were assessed as birth outcome measures. A total of 191 women were recruited for 2 prenatal visits, and their delivery information was collected from their medical charts. Statistical analyses included multiple linear regression, independent t test, chi square, and two-way ANOVA. For mothers of male babies, high cortisol levels during the third trimester were related to higher scores of stressful life events.Albeit preliminarily, the results indicate that maternal prenatal stress adversely impacts birth weight of male babies, and the consistency of cortisol levels during pregnancy is associated with birth weight of female babies. Further studies on contextualization of stress measures and improvement on statistical method are suggested.

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Associations between Prenatal Maternal Stress and Birth Outcomes

Introduction

Recent studies have generally supported connections between stress and health outcomes (S. Gennaro & M. D. Hennessy, 2003). A growing number of researchers agree that both biological and psychosocial stresses can lead to neuroendocrine reactions (Hobel, Goldstein, & Barrett, 2008). Although the question of how stress impacts physical health is widely studied, the issue of how stress affects the physical health of pregnant women and their babies only recently came to the attention of investigators (Austin & Leader, 2000). Approximately half of pregnancies in healthy adult American women are involved with either prenatal or birth complications (Lobel, 1994), which may lead to negative socioeconomic, physical, and mental consequences. The interest in maternal stress is partly driven by the fact that unfavorable conditions, such as preterm birth, were found to seriously threaten infant physical health and life, whereas the etiology remains largely unknown (Austin & Leader, 2000).

Among the various types of pregnancy complications, preterm maturity and low birth weight (LBW) are the principal predictors of newborn health conditions, and are among the most widely studied (Austin & Leader, 2000; Hobel, et al., 2008). Infants with preterm birth or LBW are associated with higher risks of mortality and morbidity (Russell, et al., 2007). These two unfavorable outcomes at birth also predict higher risks of health problems, such as hypertension and hyperlipidemia, later in life. The rate of preterm birth for pregnant women ranges from 3-4% for healthy women with good socioeconomic condition to 10-15% for women with health and socioeconomic risks (Austin & Leader, 2000). Medical cost for premature birth and LBW infants is much

higher than infants born with appropriate birth weight and gestational age (Russell, et al., 2007). The increase of medical cost is not limited to infants with extreme LBW or short gestational age, a large number of infants born with sub-clinical level of low birth weight and prematurity also account for a large portion of the total hospitalization expense. Despite preventative efforts in recent years, rates of preterm and LBW newborns have not gone down over the last decade (Mathews, Menacker, MacDorman, & Centers for Disease Control and Prevention, 2004). This partly contributes to the renewed interest in how a mother's psychological well-being may impact pregnancy and birth outcomes (Austin & Leader, 2000). However, the bulk literature on how prenatal stress is associated with preterm labor and LBW is filled with mixed results (for reviews, Alder, Fink, Bitzer, Hösli, & Holzgreve, 2007; Hobel, et al., 2008).

Operationalization of Stress

One factor that likely has contributed to the inconsistency in the research on maternal stress and pregnancy outcome is the disagreement on measurement of stress (Lobel, 1994). Researchers who are interested in assessing the impact of maternal stress on birth outcomes have attempted to conceptualize and operationalize prenatal maternal stress. Beginning from the 1970s, a large number of investigators have operationalized prenatal stress as an occurrence of major life events, state anxiety, or trait anxiety. Life events are positive or negative major life situations, which can be potentially stressful. State anxiety is a person's subjective response to stimuli that the person perceives to be stress-provoking. State anxiety is characterized by negative emotions such as fear, worry, and tenseness. Trait anxiety assesses a person's general inclination to interpret situations as dangerous and anxiety-provoking. However, construction of the concept of stress has

been inconsistent across studies, which reflects the lack of consensus among researchers on the definition of stress.

Remarkably, Lazarus and Folkman (1984) identified three major conceptualizations of stress: stimulus focused, which is most commonly measured by life events; response focused, which is often assessed by anxiety or depression scales; and stimulus-response transaction focused. Both stimulus and response based methods have their own advantages and disadvantages (Lobel, 1994). Stimulus approaches tease the predictors apart from the outcomes, and provide a more objective presentation of the situation. The primary drawback of stimulus approaches is that they are insensitive to the possible individual difference in responding to the same stressor. Stress response approaches depict individuals' phenomenological experience, which better captures how the person is influenced by certain stressful conditions. The major downside of response approaches is that they might be testing a person's stable personality traits instead of situation specific responses.

In order to overcome the disadvantage of either approach, Lazarus and Folkman (1984) advocated a third way of stress measurement, which is, to encompass multiple variables including information about the stimuli, individual's own perception of the stimuli, and his/her own responses. Lazarus' framework has largely influenced subsequent researchers who have worked on stress related scientific inquiries (Lobel, 1994). Giscombe and Lobel (2005) maintained that measures, which incorporate these different paradigms, are debatably the most reliable and valid tools to assess the concept of stress. Multivariate approaches have been found to yield some of the most supportive findings that link maternal stress to adverse birth outcomes.

Over the past several years, with the development of acceptable stress measures and with the discovery of more specific biological indexes of stress, researchers are better able to answer the question of how maternal stress may impact pregnancy and birth outcomes (Hobel, et al., 2008). Research to date implies that measures which have good reliability and validity are most commonly used for the stress indexes such as stimuli (e.g., life events, racism, work or household strain, and social support), perception of stress (e.g., perceived stress), and responses (e.g., depression and anxiety). In their recent review article, Hobel and colleagues (2008) summarized some of the most frequently used stress measurements including Life Event Inventory, State-Trait Anxiety Inventory, Edinburgh Postnatal Depression Scale, Center for Epidemiological Studies Depression Scale, and Perceived Stress Scale.

As operationalized in a variety of ways, prenatal maternal stress has been implemented as a predictor for examination of different prenatal, obstetric, and postnatal outcomes. Inspections include pregnancy complications (for recent review, Alder, et al., 2007) such as spontaneous abortion (Fenster, et al., 1995; Neugebauer, et al., 1996), preeclampsia (Hobel, Dunkel-Schetter, Roesch, Castro, & Arora, 1999; Perkins, et al., 1995), delivery complications (for review, Austin & Leader, 2000), racial disparity in pregnancy complications (Giscombe & Lobel, 2005), birth defects (Carmichael, Shaw, Yang, Abrams, & Lammer, 2007), cognitive development (Bergman, Sarkar, O'Connor, Modi, & Glover, 2007), and mental and physical health of offspring (Carsten Obel, Hedegaard, Henriksen, Secher, & Olsen, 2003; Weinstock, 2005).

Self-Report Stress Measures and Birth Outcome

Among the psychological stress measures, life events have been studied most extensively by researchers who investigated prenatal stress (Lobel, 1994). Some studies looked at large-scale, traumatic life events and their impact on pregnancy outcomes (for review, Alder, et al., 2007; Hobel, et al., 2008). For example, women who lived within 2-mile radius of World Trade Center during the 911 Attack were found to have offspring with lower birth weight and shorter gestational age (Lederman, et al., 2004). The vast majority of the maternal stress literature though, focused on individual based experience of life events.

There are mainly three ways of how life events can be assessed: total number of stressful life events, subjectively weighed impact of life events, and impact of life events objectively weighed by reviewers. For studies that recorded total number of life events, the results were mixed in that some of them suggested a positive relationship with birth weight, gestational age, or labor complications, whereas some others did not (for review, Lobel, 1994). This might be partly due to the different types of life events that were evaluated, and the timing of data collection. For objective life event weights, general evidence does not support the association with gestational age, and the implication for labor complications is mixed. There has been an increase in the usage of subjective life event weights in the recent years (Austin & Leader, 2000). In a large study of nearly 6000 women by Hedegaard et al (1996), perceived stressfulness of life events were indicated to predict premature birth, controlling for maternal age, parity, past obstetric history, marital status, drinking, and smoking. In contrast, the total number of life events was not found to be related to birth outcomes. In another large study that recruited 2432 women,

perceived life events were also connected with preterm labor, but not with intrauterine growth restriction (Nordentoft, et al., 1996). In Dole et al.'s (2003) investigation of approximately 2000 women, the total score of both subjectively weighed negative and positive events did not predict preterm delivery, but the perceived total negative events score was related to preterm birth, with participants in the highest negative events group predicting the highest rate of preterm birth. In general, life event stress, as measured by subjectively rated negative events, has been more connected with gestational age, and notably a handful of studies indicated an association between life event stress and birth weight (Hobel & Culhane, 2003). For instance, one study revealed that subjective experience of life events was negatively correlated with birth weight (Wadhwa, Sandman, Porto, Dunkel-Schetter, & Garite, 1993).

Compared to life events which are viewed as acute incidents, perceived stress is viewed as an indicator of more chronic stress. A rigorous study on 1399 Russian women tested the impact of mothers' substance use, living situation, and subjective stress on the weight of newborns, controlling for factors such as maternal education, age, occupation, marital status, and parity (Grjibovski, Bygren, Svartbo, & Magnus, 2004). The results indicated that perceived stress, along with drinking, smoking, crowdedness of living conditions, and family support were independent predictors of infants' weight. In another study with a sample size of 78 mother-neonate dyads, perceived stress, in combination with corticotrophin-releasing hormone (CRH), accounted for up to 27% of the variance of gestational age (Ruiz, Fullerton, Brown, & Schoolfield, 2001). The same group also found that increase of subjective stress over pregnancy was associated with higher risks of labor with shorter pregnancy duration (Ruiz, Fullerton, Brown, & Dudley, 2002).

Similar findings were reported by Gennaro, Shults, and Garry (2008) who studied 57 African American women and found that the group with preterm delivery had experienced higher maternal stress than the group with full term delivery. However, another research team, who also recruited African American participants, failed to find associations between perceived stress and gestational age, or between perceived stress and adjusted birth weight, after controlling for potential confounding variables such as maternal age and weight gain (Dominguez, Schetter, Mancuso, Rini, & Hobel, 2005). Instead, they found life event exposure to be strongly related to length of pregnancy. A recent study on 1602 Canadian women also failed to support a connection between perceived stress and birth outcomes, whereas body mass index, smoking, obstetric history, and maternal health were indicated as risk factors of adverse pregnancy outcomes (St-Laurent, De Wals, Moutquin, Niyonsenga, & Noiseux M, 2008).

HPA Axis and Stress

Among the array of stress responses, the function of the hypothalamic-pituitary-adrenocortical (HPA) axis has been widely examined across disciplines (Hobel, 2004). One of the primary regulators of the HPA axis is CRH, a 41-amino acid peptide. CRH is produced by the paraventricular nuclei in the hypothalamus, and it promotes the production of pituitary adrenocorticotrophin hormone (ACTH) and β-endorphin at the anterior pituitary gland. In response to stress, both CRH and ACTH act on the adrenal cortex to stimulate the secretion of circulating cortisol, a glucocorticoid (Weinstock, 2005).

As the final product of this regulatory chain, cortisol affects a variety of activities including cognition, emotion, development, and reproduction (Field & Diego, 2008). In

coordination with other stress-regulatory systems, cortisol also plays a crucial role in modulating physiological and behavioral responses under stressful conditions. High basal cortisol levels are associated with generally high stress levels and more inhibited behaviors. Elevated cortisol response also indicates the severity of one's level of stress. In general, when stress is of short duration, activation of cortisol results in inhibited secretion of cytosolic glucocorticoid and mineralocortisoic receptors. Consequently, CRH and ACTH production will be suppressed, and the suitable level of physiological factors will be rapidly restored, thus homeostasis will be resumed. However, if the stress persists over a long period of time, and if the individual is not able to adjust to the prolonged adversity, the HPA axis and the peripheral nervous system are likely to be chronically stimulated, which may increase risks of health problems, such as cardiovascular disease, diabetes, inhibition of immune system function, bone decalcification, and restrictions in bodily development (Becker, Breedlove, Crews, & McCarthy, 2002). Hyperactivity of HPA axis has also been associated with a variety of psychological problems, including anxiety, depression, drinking, smoking, and schizophrenia (for review, Arborelius, Owens, Plotsky, & Nemeroff, 1999; Charmandari, Kino, Souvatzoglou, & Chrousos, 2003; Heim, 2001; Seckl, 2001; Walker & Diforio, 1997). In addition to the duration of stress, other factors, such as genetic predisposition, timing, and intensity of stressful events, may also contribute to adverse outcomes (Austin & Leader, 2000).

HPA Axis and Pregnancy

During pregnancy, a good amount of CRH is stored in the placenta (Trainer, 2002). A large portion of plasma CRH is inactivated by the CRH bounding protein (CRH-BP) until the third trimester. When the level of the CRH-BP drops during the third

trimester, the amount of active CRH rises, and CRH in turn stimulates both local and pituitary ACTH production. Moreover, CRH plays a pivotal role in preparing the fetus for labor. Maternal and fetal cortisol levels also fluctuate during gestation. Under normal circumstances, only approximately 10% of circulating cortisol is active, and the majority of cortisol is bound to cortisol-binding globulin and thus is inactive. Beginning from the second trimester, the levels of circulating, as well as that of the free unbound cortisol, significantly increase. The surge of cortisol is related to the maternal adaption to pregnancy including fluid retention, facial plethora, striae, and the tendency for development of diabetes mellitus. The circadian rhythm of overall cortisol is consistent in pregnant women at a higher level than in non-pregnant women. During the third trimester, the level of circulating cortisol is about 2-3 times as in a non-pregnant state (Mastorakos & Illias, 2003). In the mean while, the cortisol reactivity to stress is still maintained. Placental CRH activates in a positive feedback loop to stimulate the fetal cortisol secretion, which modulates the maturation of fetus and timing of pregnancy. In addition, the plasma levels of ACTH and β-endorphin also rise near term, which indicates their involvement with parturition, and their surges are likely regulated by both the placental and pituitary CRH (Chan, et al., 1993).

Hormonal Response to Stress during Pregnancy

In addition to psychological measures, studies have also incorporated biological markers (hormones, heart rate, and blood pressure, etc.) to assess the bodily responses to prenatal maternal stress (Hobel, et al., 2008). Stress hormones such as cortisol, β-endophin and CRH have been investigated for their role in pregnancy outcomes (Alder, et al., 2007; Field & Diego, 2008; Hobel, et al., 2008; C. Obel, et al., 2005; Smith, et al.,

1990; Stancil, Hertz-Picciotto, Schramm, & Watt-Morse, 2000). It has been proposed that the HPA system mediates the relationship between psychological stress and health outcomes (Austin & Leader, 2000). Notably, animal studies have implied that prenatal maternal stress increases the risk of undesirable pregnancy and birth outcomes (Hobel, et al., 2008). Rhees and Fleming (1981) studied pregnant rats and observed that extreme maternal stress was associated with altered duration of pregnancy and lower birth weight. Subcutaneous injection of CRH to rats of 2-3 weeks of age replicated the effect of maternal stress on the well being of offspring, which suggested the involvement of HPA axis in maternal stress (Williams, Hennessy, & Davis, 1995). Acute maternal stress during late gestation also likely results in elevated plasma ACTH and corticosterone (CORT, the counterpart of primate cortisol in rodents) in both the mother and the fetus (Ohkawa, et al., 1991). The fetal brain is also sensitive to maternal stress as indicated by the observation that on Day 20 of pregnancy, acute maternal stress inhibited the secretion of CRH and β-endorphin in the pregnant rat's hypothalamus and the secretion of β-endorphin and ACTH in the pituitary of the fetus. When responding to a single stressful episode, the fetal enzyme 11 β-hydroxysteroid dehydrogenase (11βHSD) speeds up the conversion of CORT into an inert form and this ameliorates the influence of maternal HPA activity on the fetus (Weinstock, 2005). However, under the condition of chronic stress, constant electric shock to pregnant rats resulted in prolonged increase of CORT in both the mother and the fetus (Takahashi, Turner, & Kalin, 1998). The hormonal variation is associated with reduction of CORT binding globulin (CBG) which indicates elevation of active CORT. It is also possible that the fetal 11βHSD is inactivated in addition to the change of the CBG. The data support that chronic maternal stress exerts

more adverse impacts on the developing fetus than acute stress does, as there is a stronger connection between maternal and fetal plasma hormonal levels under long term stress than under short term stress (Ohkawa, et al., 1991; Weinstock, 2005).

The findings of these animal studies are consistent with the observation of human participants that undesirable pregnancy outcomes are more associated with chronic stress than acute stress (Weinstock, 2005). Human placenta is also sensitive to stressful stimulation (Hobel & Culhane, 2003). CRH in cultured placental cells is responsive to the activity of a variety of neurotransmitters and stress hormones (Petraglia, Sawchenko, Rivier, & Vale, 1987), and it also modulates the expression of ACTH in a dose-dependent manner (Petraglia, et al., 1987). Research on human subjects showed strong associations between plancental CRH levels and HPA hormones, such as ACTH and cortisol (Chan, et al., 1993; Goland, Conwell, Warren, & Wardlaw, 1992; Sasaki, Shinkawa, & Yoshinaga, 1989). Maternal psychosocial stress is found to stimulate hormones in the HPA axis (ACTH, cortisol etc.), which in turn activate plancental CRH. It is controversial whether plancental CRH level is directly regulated by maternal stress as findings are not consistent across studies. A large body of literature has implied that women with preterm delivery have much higher CRH levels and higher elevated rates of CRH than the control group. These differences become detectable several weeks before the onset of labor. High CRH levels were also reported in the cord blood of mothers with other pregnancy complications during labor (Challis, et al., 2001). These complications include hypertension, fetal asphyxia, umbilical-vascular insufficiency, preeclampsia, fetal growth restriction, and multiple births (Pike, 2005).

Among all the human HPA hormones, cortisol is one of the most extensively investigated components, and was included in the present study as a biological marker of stress. Cortisol can be found in various bodily fluids, such as saliva, urine, and blood. Salivary cortisol is suggested to be a better indicator of the HPA axis activity, because only the unbound form is present in saliva, whereas measurements based on blood and urine samples include both the free and bound forms (Field & Diego, 2008). Normally, the placenta serves as a barrier that protect the fetus from being exposed to elevated levels of maternal cortisol, as 11βHSD works to inactivate cortisol (Hobel, et al., 2008). When early maternal stress results in excessive exposure of cortisol to the fetus, or when the function of 11βHSD is impaired, fetal development will be adversely influenced by the alteration of the glucocorticoid levels.

A recent review by Field and Diego (2008) summarized the up-to-date research on the function of cortisol during pregnancy, and drew lines between high prenatal cortisol levels and pregnancy conditions including spontaneous abortion, irregularity in fetal activity, and impeded fetal growth. Problems later in life, such as conduct problems, mental disorders, and chronic health conditions, were also indicated to be at higher chances of occurrence for those whose mother endured high cortisol levels during pregnancy. In terms of birth outcome, elevated cortisol levels were found in some studies to contribute to shortened pregnancy duration, and lower birth weight (for review, Field & Diego, 2008). Pregnant women who exhibited depressive symptoms were reported to have high cortisol levels, and they also had higher risks of giving birth to preterm and LBW babies (Field, et al., 2004; Field, et al., 2006). The effect of cortisol on gestational age is implicated to take place as early as 14 weeks of pregnancy (Sandman, et al., 2006).

However, consensus is yet to be established as some other studies failed to suggest a relationship between cortisol levels and gestational age. A study by Ruiz et al. (2001) that collected blood samples at multiple time points for cortisol measurement during the second and third trimesters, did not link cortisol level to newborns' gestational age. Instead, the authors found that decrease of PSS score was associated with increase of gestational age. Another study which focused on the impact of domestic violence only supported cortisol's role in predicting fetal growth restriction, but not premature delivery (Valladares, Peña, Ellsberg, Persson, & Högberg, 2009). This raises the question of how stressors of different nature may activate the physiological system differently, and this difference may lead to diverse impacts on the fetal function. One particular example is the relationship between cortisol and pregnant women who developed post-traumatic stress disorder (PTSD). As examined in a handful of studies, in general, pregnant women who suffered from PTSD were indicated to have low cortisol profile (Seng, Low, Ben-Ami, & Liberzon, 2005; Yehuda, et al., 2005).

Hypotheses of the Current Study

The target population of the current study was minority pregnant women, mainly African Americans. The disparity between African American and Caucasian American neonate mortality rates has been widened in recent years (Hogue & Bremner, 2005). It implies a difference in stressors or vulnerability to stressors in different ethnic groups. This raises the question of whether the study design and the finding of one ethnic group are generalizable to another group. For instance, stress measures that are heavily influenced by cultural and contextual factors, particularly life event examples, might be problematic for groups with different backgrounds (Lobel, 1994). Some research suggests

that there might be difference in how well life events can be used to predict birth outcome among difference racial groups (Hobel & Culhane, 2003). The response patterns may also differ in groups whose socioeconomic status and other chronic stresses (e.g., racism) are not the same.

The timing of the stressor is also likely a factor that contributes to the difference in results (Hobel, et al., 2008). During pregnancy, the fetus experiences vast developmental changes and is very vulnerable to environmental influences (E. P. Davis, et al., 2007). Typically, stress is assessed during the second and third trimesters, and after delivery (Susan Gennaro & Mary Dawn Hennessy, 2003). It is not clearly established yet whether there is a critical period during gestation when certain types of stress might be more harmful than some others. Multiple waves of stress assessment have been widely advocated, so that the timing effect of stress response will be better understood. Some studies suggest that prenatal exposure to major traumas such as earthquakes and the 911 attack during the first trimester has more adverse impact on birth outcome than it does during later pregnancy (Glynn, Wadhwa, Dunkel-Schetter, Chicz-Demet, & Sandman, 2001; Lederman, et al., 2004). However, a study of early prenatal exposure to Ukrainian Chernobyl blast did not indicate increased risk of birth outcomes (Levi, Lundberg, Hanson, & Frankenhacuser, 1989). As for non-catastrophic stresses, it was indicated by Hoffman and Hatch's (2000) study that between late second and early third trimesters pregnant women's mental well being would have significant impact on fetal development.

As discussed earlier, the discrepancies in definition and measurement of stress partly contribute to the inconsistencies across studies (Dole, et al., 2003; Hedegaard, et al., 1996; Lobel, 1994). The present study applied Lazarus's (1984) transaction model of

stress to examine longitudinally how different indexes of stress predict pregnancy outcomes. Basically, a multivariate approach were implemented which included a combination of life event stress, chronic subjective stress, and physiological stress.

Firstly, studies have implicated a relationship among stress measures during pregnancy (Lobel, et al., 2008; Wadhwa, Dunkel-Schetter, Chicz-DeMet, Porto, & Sandman, 1996). Both number of life events and subjective rating of life events were found to be associated with perceived stress at different stages during pregnancy at moderate strength (Lobel, et al., 2008). A study by Dominguez and colleagues (2005) which was based on African American pregnant women also found an association between the number of life events and subjective stress.

The relationships between maternal stress and hormones in the HPA axis have also been assessed by researchers. Prenatal depression, anxiety, and anger were linked to high cortisol level (Field, Diego, Hernandez-Reif, Gil, & Vera, 2005; Field, et al., 2003). As summarized earlier, animal studies strongly support a HPA hormones' mediating effect between stress and pregnancy and birth outcome. Some research findings, but not all, suggest that psychological stress is associated with activities of hormones, such as CRH and cortisol, in human beings. For instance, a study which looked at pregnant Nicaragua women showed that perceived stress, along with partner violence and low social economic status, predicted high salivary cortisol level (Valladares, et al., 2009). Another study reported significant correlations between factors (such as pregnancy anxiety, life event stress, perceived stress, general and specific support), and plasma levels of ACTH, β-endorphin, and cortisol. In their study, cortisol was found to be correlated with both measures of social support, but not with any of the stress measures,

and perceived stress was found to be correlated with the levels of ACTH (Wadhwa, et al., 1996). Factors such as participants' racial origin and the level and nature of the stress that they encountered might have contributed to the difference in these findings.

Two other studies examined life events, perceived stress, and cortisol together during pregnancy, but neither of them tested the relationship between these 3 stress measures (Harville, Savitz, Dole, Thorp, & Herring, 2007; Stancil, et al., 2000). One study looked at how these stress indicators may predict bacterial vaginosis (Harville, et al., 2007), and the other study looked at predictors of stress during pregnancy (Stancil, et al., 2000). The current study tested the relationship among these 3 stress measures. Perceived life event stress was predicted to be correlated with subjective stress at the same assessment time. The relationships between the two psychological stress measures and cortisol were also examined in this study. Since cortisol has been largely indicated to continue its role as a bodily stress marker during pregnancy (Field & Diego, 2008), it was hypothesized that both life event stress and perceived stress were associated with cortisol level at the same assessment time.

Secondly, the current study examined how different stress measures acted together to impact birth outcome. As discussed earlier, there had been evidence that supported the relationships between the three types of stress measures (namely perceived objective stress, subjective stress, and biological stress) and two specific kinds of birth outcome (namely birth weight, and gestational age) respectively, although agreement has not been reached as some other studies failed to support the associations. As it was suggested that multivariate approaches revealed stronger connections between stress measures and birth outcomes (Giscombe & Lobel, 2005), in this study it was proposed

that the combination of subjective and biological stress indicators collected during the second trimester, and the combination of all the three stress measures during the third trimester, predicted birth weight and gestational age. The directions of the associations between the aggregated stress and these two birth outcomes were expected to be negative.

As for delivery complications, existing research generally support that stress, particularly in the form of state anxiety, predicted a variety of intrapartum problems (for review, Paarlberg, Vingerhoets, Passchier, Dekker, & Van Geijn, 1995). There are also different ways to measure pregnancy and obstetric outcomes. It is controversial whether to use a summary score or a specific score for each of the condition (Lobel, 1994). Some studies imply that stress may have unequal impacts on different types of delivery complications, and using an overall score might miss the complexity of etiology and presentations of various pregnancy and birth problems (Alder, et al., 2007). However, it is possible that some pregnancy and obstetric outcomes may be significantly associated with each other, and the aggregation of individual outcome scores may facilitate the investigation on general etiopathological patterns of adverse birth outcome (Lobel, 1994). Few studies had used summary score that aggregated different types of labor problems, and this study incorporated the composite approach to examine the relationship between these 3 stress indicators and birth complications. It was hypothesized that the subjective and biological stress measures collected during the second trimester, and the 3 stress measures collected during the third trimester, jointly predicted the overall obstetric complication score. The direction of the relationship was predicted to be positive.

Thirdly, the impact of change of stress levels over pregnancy on birth outcome has only lately drawn the attention of very few researchers (Glynn, Schetter, Hobel, &

Sandman, 2008; Ruiz, et al., 2002). Glynn et al. (2008) found that increase of perceived stress during pregnancy was associated with premature delivery. Ruiz et al. (2002) used the same kind of stress measure, and found a connection between increased maternal subjective stress and decreased gestational age of the fetus. The current study examined how the combination of change of perceived stress measure and biological stress marker predicted both birth weight and gestational age. As increased maternal stress might have adverse influence on fetal development, particularly since perceived stress and cortisol have been linked to both birth weight and gestational age by some studies, it was proposed that, the changes of these two stress measures were associated with birth weight and gestational age. The directions of the relationships were expected to be negative.

Method

Measures

The Life Experiences Survey (LES). The LES is a 57-item scale used for assessing life incidents that happened in the past year (Sarason, Johnson, & Siegel, 1978). It is composed of two sections: the first section contains 47 items, and it is concerned with life events that are common to an individual in a wide variety of situations. The second section contains 10 items that are only for academic experiences. Only the first section was used for the current study. Respondents were asked to rate each experienced life event on a 7-point Likert scale ranging from extremely negative to extremely positive. If an event did not occur, the item was coded as 0. Three scores are generated from the scale: the overall change score (total LES), which is the sum of all the subjectively rated events; the negative change score (negative LES), which is the sum of all the negative scores; and the positive change score (positive LES), which is the sum of all the positive

scores. As the authors noted, the test-retest reliability might not be a good indicator of the psychometric property of the scale since new events may occur during the interval. The negative experience score was found to be positively correlated with anxiety and depression measures.

The Obstetric Complications Scale (OCS). Obstetric complications were recorded by using the OCS (Littman & Parmelee, 1978). The original measurement contains both the OCS and the Postnatal Complication Scale. The current study only included the results from the OCS for analysis. The OCS assesses conditions related to maternal prenatal health and delivery complications. This measurement is composed of 41 items obtained from the medical charts, and each item is rated as optimal or non-optimal. A summary score is generated by adding up the responses of all the 41 items. High OCS total score is generally associated with adverse outcome.

The Perceived Stress Scale (PSS). The PSS is a 14-item measure, which asks subjects to rate the extent to which they have felt their life to be stressful within the last month. It measures the degree of self-appraised stress in one's life (Cohen, Kamarck, & Mermelstein, 1983). Higher total score on PSS is thought to reflect higher subjective stress. The PSS was found to have good internal consistency and construct validity (Roberti, Harrington, & Storch, 2006).

Participants and Procedures

A total of 191 women, who received their prenatal care at the Grady Hospital, Atlanta, Georgia, were recruited for 2 prenatal visits (26-28 weeks of gestation, and 32-34 weeks of gestation). The participants were compensated for each study visit $10-$40 plus a bus card or up to $5 for parking/transportation. The average age of the participants

was 23.29 years with a standard deviation of 5.5 years. Other demographics including ethnicity, social economic status, and infant sex were shown in Table 1. During the first and second visits, the participants were first required to complete some questionnaires. For the first visit, the participants filled out a form which assessed their demographic information including age, gender, ethnicity, and social economic status. They were also administered the PSS. For the second visit, they were administered both the PSS and the LES. The information of birth weight, gestational age of the newborns, and scores of OCS were obtained from their medical charts after delivery.

Afterwards, the fetuses were stimulated by a vibrator, Toitu Model TR-30 (Toitu, Japan). Their heart rate and movement were recorded by monitor MT-516 (Toitu, Japan). Saliva samples were collected from the participants once upon their arrival, and once after the fetal heart rate monitoring. Each time the participants were asked to wet a cotton stick in their mouth, and the saliva sample was squeezed from the cotton stick into a tube by a syringe. Collected saliva samples were immediately centrifuged and stored in -20 °C until analysis. The Salimetrics cortisol kit (Salimetrics, PA) and the Stat Fax 2100 (MIDSCI, MO) were used to test the saliva cortisol level. The cortisol level from the salivary samples collected before the following assessment was regarded as basal cortisol level and was included for analyses.

Statistical Analyses

The α level of the study was .05. For tests of directional hypothesis, the p values were one-tailed. Otherwise, the p values were two-tailed. One sample Kolmogorov-Smirnov test (K-S test) was used to test the normality of the continuous variables that were included in the analyses. The relationships among the three stress measures and

among the three measures of birth outcome were tested by Pearson correlation. To test the relationships between the stress indicators and birth outcome measures, multiple linear regressions, independent t test, and chi square were used. Interactions were assessed by using two-way analysis of variance (two-way ANOVA).

Results

Missing Data

For all the predictors and the dependent variables in the study, there were some missing cases. The missing data rates varied from 13.0% to 55.5% (Table 2 and 5 listed the valid cases of the variables). Since the missingness was at high rates, imputation was not an appropriate strategy to compensate for it. Instead, availability-case analysis was adopted for each of the statistical inquiries in order to optimize the power of each analysis.

Hypothesis One: Stress Measures at T1 and T2

The results of one sample Kolmogorov-Smirnov test (K-S test) showed that the total score of PSS at T1 (*K-S z* = .899, *p* = .395), the total score of PSS at T2 (*K-S z* = .721, *p* = .675), the grand total number of events (*K-S z* = .823, *p* = .508), and the total LES score (*K-S z* = 1.234, *p* = .095) were normally distributed (α = .05), whereas the distributions of the negative LES (*K-S z* = 2.459, *p* < .001, skewness = 1.509), the positive LES (*K-S z* = 1.652, *p* = .009, skewness = .715), mother's cortisol level at T1 (*K-S z* = 2.635, *p* < .001, skewness = 5.465), and mother's cortisol level at T2 (*K-S z* = 1.443, *p* = .031, skewness = 2.074) were positively skewed. The descriptive statistics of the stress measures were shown in Table 2. For the variables that were normally

distributed, means and standard deviations were listed. For the variables that were not normally distributed, medians were listed.

In order to perform parametric tests that involved the skewed variables, they were transformed into variables that were normally distributed as examined by K-S test: square root of the negative LES (*K-S z* = 1.349, *p* = .053), square root of the positive LES (*K-S z* = .744, *p* = .637), log of mother's cortisol level at T1 (*K-S z* = 1.209, *p* = .107), and square root of mother's cortisol level at T2 (*K-S z* = .940, *p* = .340). All of the parametric tests in this study that pertained to any of these variables used the transformed versions.

The results of Pearson correlations between the stress measures were illustrated in Table 4. The number of valid pairs for the intercorrelations varied from 125 to 134. Specifically, the PSS scores that were collected at the two time points correlated strongly with each other, *r* (125) = .624, *p* <.01. For the subscales of LES at T2, the transformed negative LES was found to have moderate positive correlation with PSS at T1, *r* (129) = .293, *p* <.01, and T2, *r* (128) = .307, *p* <.01, whereas the square root of positive LES was found to have moderate negative correlation with PSS at T1, *r* (129) = -.227, *p* <.01, and at T2, *r* (128) = .321, *p* <.01. The grand total number of events was only found to be correlated with PSS at T1, *r* (129) = -.202, *p* <.05, but not with PSS at T2, and the total overall change score was found to be correlated with neither of the subjective stress measures. Based on the hypotheses, the negative LES was used as the measure of life event stress, which reflected subjectively rated objective stress.

Neither of the cortisol levels at the two collection time points was correlated with any of the psychological measures. In order to examine the relationship between life event stress and biological stress at T2 in a different way, the following two variables:

negative LES at T2 and cortisol at T2 were coded into two levels respectively with median splits, which yielded a low LES condition and a high LES condition from the negative LES variable, and a low cortisol condition and a high cortisol condition from the cortisol variable. The result of chi-square test of independence suggested a trend of association between high maternal cortisol and high negative LES score at T2, X^2 (1, N = 83) = 2.704, p = .100. Interestingly, when these participants were divided into two groups: those who had male babies and those who had female babies, the results of chi square test of independence indicated that there was a significant association between mother's cortisol level and the negative LES score for the male fetal group, X^2 (1, N = 40) = 4.000, p = .046. The number of participants who had both high cortisol levels and high negative total change scores, and the number of participants who had both low cortisol levels and low negative total change scores were larger than the expected counts (Table 3). For the female fetal group, the chi square test result was nonsignificant, X^2 (1, N = 40) = 0.00, p = 1.000. Similar non-parametric analyses did not show connections between cortisol levels and PSS at either time point.

Birth Outcomes

The results of one sample K-S test showed that birth weight (*K-S* z = 1.273, p = .078) and OCS total score (*K-S* z = 1.209, p = .107) were normally distributed, whereas the distribution of gestational age (*K-S* z = 1.696, p = .006) was negatively skewed. The descriptive statistics of the birth outcome variables were shown in Table 5. For the variables that were normally distributed, means and standards deviations were listed. As gestational age was not normally distributed, median was listed. In order to perform parametric tests that involved gestational age, it was transformed into the variable that

was created by first subtracting each gestational age from the maximum gestational age of 41.71 weeks, and then by square rooting the difference. The transformed variable, named as transformed gestational age, was normally distributed as indicated by K-S test (*K-S z* = 1.195, *p* = .115). All of the parametric tests in this study that included gestational age used the transformed variable. Because of the nature of the transformation, if significant results were detected for transformed gestational age, the direction of the relationship between gestational age and the other variable(s) would be reversed.

The result of Pearson correlation showed that birth weight had a negative and strong association with transformed gestational age, r (88) = -.683, $p < .001$. Both of these variables were also correlated with OCS total score. For birth weight: r (88) = -.577, $p < .001$, and for transformed gestational age: r (88) = .496, $p < .001$.

Hypothesis Two: The Relationships between Stressors at T1 and Birth Outcome

Three multiple linear regressions were conducted to examine the relationships between stress variables at T1 and birth outcome variables. Birth weight, transformed gestational age, and OCS total score were respectively regressed against PSS and log of mother's cortisol at T1, controlling for time of saliva collection. Results did not show linear associations between these two stress indicators and any of the outcome variables (the degrees of freedom of these three regressions ranged from 32 to 43).

When maternal cortisol at T1 was coded into high cortisol and low cortisol levels based on the median split, the result of independent t test revealed that mothers with high cortisol levels had lower OCS total scores (M = 3.89, SD = 2.52) than mothers with low cortisol levels (M = 6.42, SD = 2.22), t (35) = 3.250, p = .003. When fetal sex was tested for its interaction with cortisol level, a two-way ANOVA indicated a trend for the

interaction term, F (1, 31) = 3.345, p = .077. These participants were further divided into two groups: those who gave birth to male babies, and those who gave birth to female babies. For the male fetal group, the OCS total scores of the 2 groups with different cortisol levels did not differ from each other, t (16) = 1.006, p = .329. For the female fetal group, mothers with high T1 cortisol had lower OCS total scores (M = 3.10, SD = 2.03) than mothers with low cortisol (M = 7.14, SD = 2.67), t (15) = 3.558, p = .003.

In order to further determine how these two stressors might act together to impact birth outcomes, a new variable was created which had 4 levels: low PSS, low cortisol (LPLC); low PSS, high cortisol (LPHC); high PSS, low cortisol (HPLC); high PSS, high cortisol (HPHC). The coding was based on the mean of PSS score at T1 and median split of cortisol value at T1. The results of one-way analysis of variance (one-way ANOVA) did not suggest difference among participants with the 4 conditions for birth weight (F (3, 40) = .205, p = .892), transformed gestational age (F (3, 38) = .539, p = .659), or OCS total score F (3, 29) = 2.911, p = .051).

To further examine whether there was an aggregation effect of the two stress measures on birth outcomes, the HPHC level was kept separately as one high stress condition, and the remaining LPLC, LPHC, and HPLC levels were combined together as one combined low stress condition. The results of independent t test did not show difference in birth weight (t (42) = 1.086, p = .141), or OCS total score (t (31) = .193, p = .425) between participants with 2 stress levels. There was a trend that the high stress group had babies with shorter gestational age (t (35) = -1.655, p = .054). Since it has been reported that male newborns tend to have larger body size and higher birth weight than female newborns (McGregor, Leff, Orleans, & Baron, 1992), fetal sex was assessed

as an additional factor for the nature of the relationship. A two-way ANOVA was conducted to test whether fetal sex was a moderator between these 2 stress levels and birth weight. The interaction was not statistically significant, F (1, 38) = .262, p = .612. However, the results of independent t test showed that women in the high stress condition had male babies with lower birth weight (M = 3119.38, SD = 170.49) than those in the combined low stress condition (M = 3380.36, SD = 311.41), t (20) = 2.176, p = .021 (also shown in Table 6). No differences in maternal age, SES, ethnicity, saliva collection time, or babies' gestational age were found between the two male fetal groups. No difference between these two stress conditions was shown for the participants with female babies, t (18) = .233, p = .410.

Hypothesis Two: The Relationship between Stressors at T2 and Birth Outcome

In order to examine the associations between stress indicators at T2 and birth outcome variables, birth weight, transformed gestational age, and OCS total score were respectively regressed against PSS score, square root of negative LES, and square root of mother's cortisol at T2, controlling for time of saliva collection. The results failed to indicate linear associations between the 3 stress measures and any of the birth outcome variables (the degrees of freedom of these three regressions ranged from 31 to 45).

With the purpose of further investigating how stress life events and biological stress might act together to influence birth outcomes, a new variable was created that had 4 conditions: low negative LES score and low cortisol (LNLC); low negative LES score and high cortisol (LNHC); high negative LES score and low cortisol (HNLC); high negative LES score and high cortisol (HNHC). The classification was based on the median splits of negative LES and cortisol at T2. The results of one-way ANOVA failed

to reveal any difference among the groups for birth weight (F (3, 47) = 2.434, p = .077), transformed gestational age (F (3, 44) = .235, p = .871), or OCS total score (F (3, 32) = .702, p = .558).

To further test the aggregation effect of these two stressors, the HNHC level was remained as a high stress condition, and the LNLC, LNHC, and HNLC levels were combined together and re-coded into a low stress condition. The result of independent t test did not support a difference between participants with these two stress levels in birth weight, t (49) = -.719, p = .238. A two-way ANOVA was conducted to test whether there was an interaction between baby sex and the 2- level stress variable in predicting birth weight, gestational age, and OCS total score. Interaction was only found for birth weight, F (1, 46) = 5.107, p = .029. The participants were then split into a male group and a female group based on fetal sex, to test respectively the relationship between the 2-level stress condition and birth weight. Interestingly, the female group in the high stress condition had babies with higher birth weight (M = 3278.12, SD = 528.43) than participants in the low stress condition (M= 2826.43, SD = 398.39), t (20) = -2.104, p = .024 (also shown in Table 6). No differences in maternal age, SES, ethnicity, saliva collection time, or babies' gestational age were found between the two female fetal groups. For the male group, no difference in birth weight was found between the two conditions, t (26) = .791, p = .118.

To examine the aggregation effect differently, the LNLC level was kept as a low stress condition, and the LNHC, HNLC, HNHC levels were combined together to form a high stress condition. Independent t test revealed higher baby birth weight in the participants with low stress condition (M = 3357.00, SD = 415.53) than those with high

stress condition (M = 3120.76, SD = 458.864), t (49) = 1.815, p = .038. Although the result of a two-way ANOVA did not support an interaction between fetal sex and the 2-level stress condition, F (1, 46) = 1.855, p = .180, for the male fetal group, participants in the combined high stress condition had babies with lower birth weight (M = 3245.33, SD = 233.24), as compared to participants in the high stress condition (M = 3481.62, SD = 363.96), t (26) = 2.077, p = .024 (also shown in Table 6). No differences in maternal age, SES, ethnicity, saliva collection time, or babies' gestational age were found between the two female fetal groups. For the female fetal group, no difference was found between participants under these two conditions, t (20) = -.491, p = .315. No aggregation effect was found for the combination between PSS and cortisol, or between PSS and negative LES score.

Hypothesis Three: The Relationship between Stressors at T1, T2 and Birth Outcome

Two new variables, namely PSS change and cortisol change, were created respectively by subtracting participants' PSS score at T1 from their PSS score at T2, and by subtracting participants' cortisol value at T1 from their cortisol value at T2. The results of one sample Kolmogorov-Smirnov test (K-S test) showed that the PSS change (K-S z = 1.113, p = .168) was normally distributed, whereas the cortisol change (K-S z = 2.368, p < .001) was negatively skewed. However, transformations of cortisol change via square root, log 10, or reversion after being subtracted from a constant did not result in normal distribution. For the following multiple linear regressions that involved cortisol change, assumption of normality for the distribution of this variable was violated.

Two multiple linear regressions were conducted to test the relationships between PSS change, cortisol change, and two birth outcome variables. Birth weight and

transformed gestational age were respectively regressed against PSS change and square root cortisol change. The variance of birth weight accounted for by the two predictors was .4%, and the variance of transformed gestational age accounted for by the two predictors was 2.3%. The results showed no linear associations between the two variables and the two birth outcomes (the degrees of freedom of the regressions ranged from 26 to 28).

Based on the median splits of cortisol values at the two time points, a new variable was created that had four conditions: low cortisol at T1 and low cortisol at T2 (LCLC), low cortisol at T1 and high cortisol at T2 (LCHC), high cortisol at T1 and low cortisol at T2 (HCLC), and high cortisol at T1 and high cortisol at T2 (HCHC). The results of one-way ANOVA did not show difference among the 4 groups for birth weight (F (3, 33) = 2.223, p = .104) or transformed gestational age (F (3, 30) = .960, p = .425). Although the omnibus F for OCS was significant (F (3, 22) = 3.703, p = .027), the post hoc turkey test did not show any two groups that were significantly different from each other. The LCLC and HCHC levels were combined and further re-coded into a consistent condition, and the LCHC and HCLC levels were combined and re-coded into an inconsistent condition. A two-way ANOVA was used to examine the interaction between baby sex and cortisol consistency level, and the results showed that there was an interaction between baby sex and the cortisol consistency level, F (1, 32) = 6.186, p = .018. The participants were then categorized into a male group and a female group based on fetal sex. For the male group, there was no difference in babies' birth weight between the consistent group and the inconsistent group, t (19) = .100, p = .921. For the female group, the consistent group had babies with higher birth weight (M = 3357.14, *SD*

= 539.34) and gestational age (M = 39.10, SD = 1.29) than the inconsistent group (birth weight: M = 2673.75, SD = 401.67; t (13) = 2.808, p = .015; gestational age: M = 36.91, SD = 2.18; t (13) = 2.32, p = .037). No differences in maternal age, SES, ethnicity, or saliva collection time were found between the two female groups.

Discussion

Hypothesis One: Stress Measures at T1 and T2

As predicted by the hypothesis, the correlations between perceived stress and life event stress (i.e., negative LES) were found to be significant during the early third trimester. This was supported by the maternal stress literature (Dominguez, et al., 2005; Lobel, et al., 2008). Generally, a moderate and positive relationship was found between subjective stress and life event stress. Notably, the majority participants in the current study self-identified as African Americans with middle or lower middle socioeconomic status, and participants in Dominguez's (2005) study shared similar social economic status and ethnic composition. The results in this study regarding the relationship between life event stress and perceived stress were consistent with the findings by Dominguez and colleagues (2005). Furthermore, when assessments of perceived stress were approximately one month apart, strong correlation was found between the total scores during the two collection time points. This suggests that the measurement of perceived stress has good consistency across time, and can be used as a chronic subjective stress indicator. Life event stress, which occurred within last 12 months, assessed during the third trimester, were also found to be related to subjective stress during the second trimester. This correlational pattern implies that recent unpleasant

acute life incidents may have an enduring effect on individuals' perception of his/her general stress level.

In addition, the total positive change score, i.e., measurement of perceived positive life events, was negatively correlated with perceived stress both at T1 and T2. For the perceived total life events with summation of both positive and negative scores, there was no correlation with perceived stress levels at either data collection time points. This indicates that life experiences that are positively perceived are not likely to be as stress provoking as life experiences that are negatively viewed. Instead, the negative relationship between total scores of positive life events and perceived stress suggests that experience of pleasant life events functions to reduce individual's feelings of stressfulness. As the effects of positive life experience and negative life experience on individuals' perception of stress appeared to counteract each other, it followed that perceived total life events were not related to perceived stress.

The implication of the findings for stress research is that when life events are included as one aspect of stress, researchers need to make sure that the life events that they analyze are stress-provoking to the participants. As the same life incident may mean differently to different people in terms of the nature and intensity of the stressfulness, their subjective ratings of specific events will help clarify the stressfulness of each event to each participant. With regard to the term "life events", since it does not exclude the experiences which are positive or neutral, and as positive events may confound the operational definition of stress, "stressful life events" may serve as a better reflector of perceived objective stress. Some earlier publications failed to specify such differentiation

(Lobel, 1994), whereas some more recent studies include the participants' ratings of the level of distress of their experience (Dominguez, et al., 2005; Lobel, et al., 2008).

Pearson correlations between coritisol levels and the two psychological measures were nonsignificant. This is inconsistent with the findings by Valladers et al. (2009). In their study, perceived stress, as well as partner violence and low social resource, was related to cortisol levels in a group of women in Nicaragua. A closer examination of their study showed that the saliva samples were collected over a wide range of pregnancy time points from 18 to 39 weeks, and the collection time was restricted to one hour in the morning and one hour in the afternoon. The average salivary cortisol value ranged from .3 µg/dl during morning collection to 1.01 µg/dl during afternoon collection. In comparison, in the current study salivary samples were gathered between 26 and 34 weeks of pregnancy, and the collection time was scattered throughout the day. The medium salivary cortisol values in the present study were .20 µg/dl during the second trimester, and .25 µg/dl during the third trimester. The central tendency of salivary cortisol levels in the current study is consistent with previous pregnancy cortisol literature (E. P. Davis, et al., 2007; Harville, et al., 2007). Since the distribution of number of women across gestational weeks during the data collection time was not specified in Valladers et al.'s paper, it is uncertain whether the high cortisol profile in the Nicaraguan participants was due to a high proportion of them at later gestational stage. In addition to the alternative explanation that cultural and ethnic factors might have contributed to the difference in these findings, it is also likely that the Nicaraguan participants were generally exposed to higher stress than the participants in this study. High occurrences of partner violence in various forms might have increased the Nicaraguan women's baseline

salivary cortisol levels. In addition, although the majority of participants in both studies were from low social economic background, the Nicaraguan women might have faced more financial and political challenges in their daily lives. Exposure to higher levels of stress might have increased their HPA system's responses to psychological stress during pregnancy. It is also likely that restricting the saliva collection time in Valladers et al.'s (2009) research successfully controlled the time effect on cortisol level fluctuation due to the time of the day, and this might have contributed to their positive findings.

The present study though, was in line with the findings in Wadhwa et al.'s (1996) study that there were no correlations between cortisol levels and life event distress or perceived stress. Although Wadhwa et al.'s (1996) study tested blood cortisol levels instead of salivary cortisol levels at 28 weeks of gestational age, and the racial composition in their study was very different from this study, it also failed to support a linear relationship between cortisol and life event distress or perceived stress. However, a non-parametric test conducted in the present study revealed a trend of association between high life event stress scores and high cortisol levels. Interestingly, the relationship was significant for mothers of male babies, but not for mothers of female babies. These results indicate that perceived objective stress might be associated with cortisol activity in a non-linear way, and conception with male fetuses might increase maternal physiological reactivity to stress exposure.

Hypothesis Two: The Relationship between Stress Measures and Birth Outcomes

For Hypothesis Two, the three multiple linear regressions that tested the relationships between the two stress measures during the second trimester and the three birth outcome indices were nonsignificant. The next three multiple linear regressions that

looked at the relationships between the three stress measures during the third trimester and the three birth outcome indices were also nonsignificant. This indicates that there are no linear relationships between these stress measures and any of the birth outcome variables. However, further categorizations and analyses suggest some connections between combined maternal stress measures and birth weight. Specifically, for mothers of male babies, low subjective stress levels and low cortisol values during the second trimester predicted higher birth weight, and high perceived objective stress levels and high cortisol levels during the third trimester predicted lower birth weight. Unexpectedly, for mothers of female babies, low perceived objective stress levels and low cortisol values during the third trimester predicted lower birth weight. It was also unforeseeable that high cortisol levels during the second trimester were associated with low OCS total scores for mothers of female babies. These patterns that existed for mothers of female babies were not shown for mothers of male babies. Further interpretations and implications of fetal sex specific results were presented in a separate section on sex dimorphism.

As discussed earlier, the maternal stress literature is filled with conflicting findings. Factors that have contributed to the complexity of these findings include, but are not limited to, operationalization of predicting variables and dependent variables, statistical analyses that are chosen, power of analyses, variables that are included to control for, nature of stress, timing of measurement, and characteristic difference among participants in different studies. As for operationalization of birth outcome, birth weight and gestational age are the measures that have been most extensively studied. Since newborns with clinically significant LBW or preterm birth are at high risks of adverse

developmental outcome (Russell, et al., 2007), LBW and premature birth have been frequently used as outcome variables by researchers. Since the base rates for LBW and preterm birth are very low, studies that have large sample size are well suited for inquiries pertained to these conditions. Usually nonparametric approaches, such as odds ratio, chi square, or logistic regression, have been implemented for analysis associated with these variables. These statistical methods are less powerful than parametric tests, but can examine nonlinear relationships for categorical variables. Some of these studies, as previously reviewed, showed some evidence of the associations between stress and these unfavorable birth outcomes (Field & Diego, 2008).

Restricted by the available number of participants, the current study did not include preterm birth or LBW as outcome variables. Instead, linear regressions were applied at first to assess the linear relationships between the combination of stress measures and birth outcomes. The results of multiple linear regressions in the present study were nonsigificant. This is inconsistent with some positive findings that examined individual stress measures and birth outcomes. Some studies in the past have indicated connections between each of these stress measures and birth weight or gestational age. For example, Wadhwa et al.'s (1993) study on 90 women revealed a negative association between life event stress and birth weight. Perceived stress has also been related to birth weight (Grjibovski, et al., 2004) and gestational age (Ruiz, et al., 2001) linearly. It is possible that for the linear regressions in the present study, low degree of freedom in these analyses that resulted in low power might have limited detection of significant results. Driven by the consideration on a trade off between degree of freedom and confounder adjustment, only saliva collection time was included as a factor to control for

in these linear regressions. The drawback of not including controlling variables is that if significant results are detected, it is uncertain whether the positive findings are due to the effect of the predictors, or are caused by other factors.

Contradictory to significant findings in above studies though, a large investigation on about 1600 participants does not support a direct association between perceived stress and birth weight, or pregnancy duration (St-Laurent, et al., 2008). Another investigation that looked at blood cortisol levels multiple times during pregnancy did not show associations between cortisol levels at any data collection time and gestational age (Ruiz, et al., 2001). Notably, a study on African American women also fails to indicate that birth weight or gestational age is predicted by perceived stress levels, although it implicates a relationship between experience of life event stress and gestational age (Dominguez, et al., 2005).

One possible explanation that accounts for the discrepancy across these studies is the difference in choice of variables to control for in their multivariate models. Among these studies that aimed at testing the relationships between stress and birth outcomes, some factors were commonly used as potential confounding variables to control for, such as education, social economic background, and some biomedical factors including maternal age and parity. Interestingly, those studies that did not link perceived stress directly with either gestational age or birth weight generally adjusted for smoking (Dominguez, et al., 2005; St-Laurent, et al., 2008). Consistently, two other large population based studies that used categorical birth outcome measures found that smoking was related directly to LBW (Jacobsen, Schei, & Hoffman, 1997; Nordentoft, et al., 1996). In contrast, life event distress has been generally indicated as a predictor of

gestational age, even after adjustment for factors including substance use (for review, Austin & Leader, 2000).

Hypothesis Three: The Relationships between Stressor Changes and Birth Outcomes

As for Hypothesis Three, the two linear regressions that looked at how the variations of perceived stress and cortisol levels during pregnancy impacted birth weight and gestational age fail to implicate linear connections between these two predicting variables and birth outcomes. However, mothers of female babies whose cortisol levels were consistent along the 2 trimesters had babies with higher birth weight and gestational age.

The question of whether the change of maternal stress levels may have any influence on birth outcomes has only been inquired lately in very few studies (Glynn, et al., 2008; Ruiz, et al., 2002). In the two studies that looked at the relationship between subjective stress and birth outcome, increased stress during pregnancy was associated with shortened gestational age. Specifically, the research by Ruiz et al. (2002) used a parametric approach to assess the linear relationship between the predictor and the outcome variable. The results of the present study were not in line with the findings of these two studies. Besides the limitation set by the available number of cases that were included for analyses, which might have contributed to the findings of nonsignificance, one big difference between the current study and these two studies was the time interval between the assessments of perceived stress. The present study collected data during the late second trimester (26-28 weeks) and the early third trimester (32-34 weeks), and the interval for each individual participant was approximately one month. In comparison, perceived stresses were measured with longer intervals for these two studies with the first

data collection time approximately 8-11 weeks earlier than the present study. It is possible that aggravation of stress over a long duration of time during pregnancy may cause more adverse consequences on gestational age. However, it is unknown whether the effect was instead due to a higher magnitude of stress increase, as relevant information is not available in these articles.

Hypothesis One, Two and Three: Sex Dimorphism

Interestingly, this study showed some fetal sex specific results for stress exposure and birth weight. The positive associations between life event stress and cortisol levels were only found for mothers of male babies. Aggregated stress appears to exert adverse impact on the growth of male fetuses. For female fetuses, it implies that the consistency of physiological stress over pregnancy, instead of the intensity of it, predicts unfavorable birth outcome.

As for male fetuses and infants, it has been observed that they are at higher risks for mortality and morbidity than female counterparts (Elsmen, Steen, & Hellstrom-Westas, 2004). Spontaneous abortion is more frequently recorded for male embryos. Higher occurrences of premature birth have been found for male fetuses, and both preterm and full-term male newborns are more likely to develop health problems. It has been proposed that the fetal and newborn male disadvantage might be associated with a higher vulnerability of male offspring to stressful stimuli. For instance, prenatal exposure to maternal smoking was linked to a higher magnitude of stress reactivity in male infants (Schuetze, Lopez, Granger, & Eiden, 2008). Another study that examined infant response to mildly stressful stimuli also revealed higher reactive cortisol change in male newborns (M. Davis & Emory, 1995). Along the similar lines, recent animal research suggests that

male infant rats are more sensitive to physical pain induced by formalin (Butkevich, Barr, & Vershinina, 2007), and display more aggressive behavior and show more brain structure change after mother-infant separation (Spivey, et al., 2009).

The greater health risks and stress vulnerability that are associated with male offspring appear to continue after infancy (Elsmen, et al., 2004). For example, the World Trade Center attack, an example of acute and traumatic stress, was linked to more behavioral problems in boys of mothers who met diagnostic criteria for depression and PTSD (Nomura & Chemtob, 2009). A longitudinal study that looked at developmental profile of Swedish children indicates that chronic psychosocial stress in home environment that is less catastrophic may also have more adverse impact on boys (Nordberg, Rydelius, & Zetterström, 1991). Another study that compared boys and girls on their spatial skill suggests that the chronic stress associated with unfavorable socioeconomic background affects boys more severely, and eliminates the male advantage that is shown for children from more favorable socioeconomic background (Levine, Vasilyeva, Lourenco, Newcombe, & Huttenlocher, 2005).

It has been speculated that the difference in male offspring's stress reactivity might be regulated by hormonal activities (M. Davis & Emory, 1995). Shortly after conception, the Y chromosome in male embryo stimulates the secretion of gonad, which fosters the development of testis (Elsmen, et al., 2004). Testosterone is generated by testis to direct the growth of secondary male characteristics. For female embryos, absence of gonad and testosterone stimulation results in development of ovary and female genitalia. Androgen is also likely involved in early fetal development, and contributes to boys' heavier weight and larger size. The congenital difference in hormonal equipment

may serve as the underlying biological mechanism that leads to greater vulnerability in boys. This is supported by the finding that there was sex difference in the correlations between cortisol and sex hormones in infants (Furuhashi, et al., 1981). During early development, the male hormonal profile may cause boys to be more susceptible to environmental stress, and consequently they might be more likely to have unfavorable birth outcomes.

Unexpectedly, for mothers of female babies, low negative LES scores and low cortisol levels at T2 were associated with low birth weight of the babies. Low cortisol levels at T2 were also linked to low OCS total scores for this group. These findings imply that the function of maternal cortisol for mothers of female offspring during pregnancy may differ from its function for mothers of male offspring. As discussed earlier, the level of maternal circulating cortisol during the third trimester is significantly higher than the non-pregnant stage (Mastorakos & Illias, 2003). The high amount of cortisol helps women better adjust to the bodily changes in response to pregnancy. For mothers of female babies in this study, high cortisol levels may reflect successful adaptations to pregnancy, and may not serve as signals of stress. There has been some evidence that supports sex difference in physiological responses to stress (for review, Kajantie & Phillips, 2006). Specifically, the HPA axis responses were found to be lower in women than in men, and the difference was detected as early as in newborns (M. Davis & Emory, 1995). The general stress levels that the women in the present study experienced might not be serious enough to markedly impact the growth of the fetuses. Interestingly, consistency of maternal cortisol levels during pregnancy appears to be predictor of birth weight and gestational age for female fetuses, but not for male fetuses. This indicates that

female fetuses are susceptible to the change of maternal cortisol levels during gestation. However, the results are based only on a sample size of 15, and a larger sample size is needed for test whether the findings are duplicable.

Notably, the OCS total score is a summary score that encompasses a variety of adverse prenatal and delivery complications (Lobel, 1994). The heterogeneity of OCS poses the question of whether different obstetric problems share similar underlying mechanisms that would lead to comparable response to stress (Alder, et al., 2007). For instance, newborns that are over-weight might be associated with maternal medical conditions such as diabetes and hypertension (Doherty & Norwitz, 2008), but on the OCS as far as birth weight is above 2500 gram, it is coded as a favorable condition. In addition to the problem related to the diversity of complication problems, the OCS is also prone to criticism for its inclusion of prenatal conditions (Lobel, 1994). Given the complexity associated with the nature of the OCS total score, it is unclear whether the association between OCS and cortisol level reflect true negative connections between maternal biological stress indicator and obstetric problems.

Theoretical models have been developed to account for the sex difference in vulnerability (Wells, 2000), and among them the evolutionary paradigm proposed by Trivers and Willard (1973) has been widely cited. In a nut shell, the model suggests that natural selection of maternal strategies to optimize reproductive success can account for the discrepancy. Generally for vertebrates, all females have good chance to mate, and for them reproductive success is achieved through producing the healthy offspring, whereas robust males have a better chance to mate, as compared to their less vigorous peers. Consequently, mothers in favorable conditions have more male offspring, and mothers in

adverse conditions have more female offspring. This theory implies that male offspring are more vulnerable than female counterparts under the influence of stress, and the disadvantage is not limited to male neonates who are born prematurely.

One implication from the sex specific results is that it is important to adjust for baby sex when examining questions pertained to maternal stress and birth outcomes. It has been well accepted that male fetuses have heavier body weight and size than female fetuses with the same gestational age (McGregor, et al., 1992). Not only offspring of different sex may react differently to environmental stimuli, fetal RNA can cross the placental barrier in small amount to affect the maternal physiological system, and thus may lead to difference in pregnant women's stress reactivity (Bianchi & Lo, 2001). Sex difference in response to prenatal stress has been suggested by some studies (Alder, et al., 2007; Kaiser & Sachser, 2005; Schuetze, et al., 2008). However, only a few studies that explored how prenatal stress influences on birth outcome included fetal sex as a potential confounder to control for, and this may pose threat to the validity of the causal conclusions in these correlational studies.

Limitations and Future Implications

It is important to note some of the limitations of this study. Firstly, some data were missing for each of the variables of interest. This was partly due to attrition, which is a common problem for longitudinal studies. The missingness not only impaired the power of data analyses, but also posed threat to the representativeness of the remaining sample. In dealing with the missing data problem, the current study took the available case analysis approach, where different subsets of questions were assessed by using different subsets of the data. The advantage of this approach is that it maximized the

sample size for each analysis. However, the potential problem of this approach is that different analyses based on different subsets of the data may not be consistent with each other. Future studies with the same longitudinal nature should consider implementing appropriate strategies to decrease subsequent attrition rate. For example, emails and phone calls right before each planned assessment time can be arranged to remind the participants.

In addition, as discussed earlier, restricted by the paucity of sample size, the multiple regressions in current study did not control for factors such as maternal age or socioeconomic status, and this might have confounded the relationship assessed between maternal stress and birth outcome. It has been advocated that information including maternal age, socioeconomic status, past obstetric history, medical condition, and substance use should be controlled for as potential confounders (Austin & Leader, 2000). There has also been some evidence that physical labor, long standing, and nutrition intake are associated with pregnancy outcome (Hobel & Culhane, 2003). Future studies should document these conditions to assist inquiries of the relationships between specific predictors and outcome measures.

As for measurement of maternal stress, expansions of stress definition that include contextual considerations have been proposed recently (Hobel & Culhane, 2003). The experience associated with conception itself can be stress provoking. A group of researchers focused on a population of low socioeconomic status in California developed a simple 4-item measure of pregnancy anxiety, which assesses fears associated with pregnancy (Dunkel-Schetter, 1998). In their study, pregnancy anxiety was found to account for preterm delivery than other measures, including state anxiety, perceived

stress, and life events. In addition, a broader sociocultural framework has been embraced for the definition of maternal stress (Hobel & Culhane, 2003; Hobel, et al., 2008). For investigation of the group of people who live in a disadvantaged neighborhood, an allostatic approach was advocated, which focuses on a chronic impact of psychosocial stress that starts earlier than conception. Factors such as racism and disadvantaged living environment maybe part of the allostatic burden that increase the likelihood of unfavorable reproductive outcomes. For instance, African American women are more likely to give birth to LBW babies if they live in a socioeconomically unfavorable community, disregard their income levels (Rauh, Culhane, & Hogan, 2000). Future studies can explore more on these contextual factors both at individual and societal levels.

In addition, the advancement in statistical methods also shed some light on the future direction in studying maternal stress. Latent variable analysis has been applied to extract smaller number of latent variables from a group of stress measures (Lobel, 1994). A recent study by Lobel et al. (2008) using structural u7equation model showed that a latent pregnancy-specific stress factor was a better predictor of both gestational age and birth weight than other more general latent stress factors, after adjustment for obstetric factors.

Studies that look at the etiopathological patterns of reproductive health are not limited to the presence of stress. The quantity and quality of social support have also drawn recent attention among investigators (Feldman, Dunkel-Schetter, Sandman, & Wadhwa, 2000; Rini, Dunkel-Schetter, Wadhwa, & Sandman, 1999). Among the studies that looked at the relationship between social support and birth weight, an association was shown between good social resources and better fetal growth. It was proposed that

social support does not only help women cope better with stress, but also may promote their healthier behavior, and provide better prenatal care to their offspring (Feldman, et al., 2000). For future studies, it will be interesting to incorporate both measures of stress and social support, and test whether there might be a few latent variables derived from all these measures that might account for pregnancy outcome better than individual measures.

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Table 1

Demographic Information of Participants

		Frequency	Percent	Valid Percent
Ethnicity				
	African American	158	82.7	83.2
	Hispanic American	26	13.6	13.7
	Caucasian American	2	1	1.1
	Asian American	1	0.5	0.5
	Mixed	3	1.6	1.6
	Valid	190	99.5	100
Social Economic Status				
	Upper	3	1.6	2
	Upper Middle	6	3.1	4
	Middle	32	16.8	21.3
	Lower Middle	63	33	42
	Lower	46	24.1	30.7
	Valid	150	78.5	100
Infant Sex				
	Male	93	48.7	52
	Female	86	45	48
	Valid	179	93.7	100

Table 2

Descriptive Statistics of Stress Measures

	N	Minimum	Maximum	Mean	Std. Deviation	Median
PSS at T1	166	5	47	24.20	7.77	--
PSS at T2	129	4	43	22.45	8.11	--
Life Event Scale at T2						
Negative LES	134	0	39	--	--	4
Positive LES	134	0	36	--	--	10
Total LES	134	0	59	19.59	11.84	--
Saliva Cortisol at T1 (µg/ml)	87	.01	3.25	--	--	.20
Saliva Cortisol at T2 (µg/ml)	85	.07	1.27	--	--	.25

Table 3

Distribution of Negative LES Groups in Cortisol Level Groups for Women with Male Fetuses

		Negative LES		Row Total
		Low	High	
Mother's Cortisol at T2	Low	18	7	25
	High	6	9	15
Column Total		24	16	40

Table 4

Intercorrelations between Stress Measures

	1	2	3.1	3.2	3.3	4	5
1. PSS at T1	--	.624**	.086	.293**	-.227**	-.069	-.096
2. PSS at T2		--	.005	.307**	-.321**	-.085	-.091
3. LES at T2:							
3.1. Total LES			--	.663**	.691**	.064	.067
3.2. Square Root of Negative LES				--	.027	-.052	.025
3.3. Square Root of Positive LES					--	.195	.072
4. Log of Mother's Cortisol at T1						--	.149
5. Square Root of Mother's Cortisol at T2							--

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 5

Descriptive Statistics of Birth Outcomes

	N	Minimum	Maximum	Mean	SD	Median
Birth Weight (Gram)	96	955	4190	3032.42	607.60	--
Gestational Age (Week)	93	28.57	41.71	--	--	39
OCS Total Score	82	0	16	5.23	3.03	--

Table 6

Means and Standard Deviations of Birth Weight of the Groups Tested for the Effects of Aggregated Stresses with Significant Results

		Male		Female	
		Mean	SD	Mean	SD
T1	Low PSS, Low Cortisol; Low PSS, High Cortisol; Low PSS, High Cortisol	3380.36 (14)	311.41	3044.53 (17)	618.86
	High PSS, High Cortisol	3119.38 (8)	170.49	2958.33 (3)	112.73
T2	Low Negative LES, Low Cortisol; Low Negative LES, High Cortisol; High Negative LES, Low Cortisol;	3382.67 (21)	337.57	2826.43 (14)	398.39
	High Negative LES, High Cortisol	3272.14 (7)	254.36	3278.12 (8)	528.43
	Low Negative LES, Low Cortisol;	3481.62 (13)	363.96	2872.50 (4)	193.63
	Low Negative LES, High Cortisol; High Negative LES, Low Cortisol; High Negative LES, High Cortisol	3245.33 (15)	233.24	3016.94 (18)	571.35